

Developmental Changes in Ionic and Osmotic Regulation in the Dungeness Crab, *Cancer magister*

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Abstract. The ontogeny of osmoregulation and specific ion regulation was studied in the megalopa, 1st instar juvenile, 5th instar juvenile and adult of *Cancer magister*. Hemolymph Na⁺, Cl⁻, K⁺, Mg⁺⁺, and Ca⁺⁺ concentrations and osmolality were measured after 8-h exposure to 100%, 75%, and 50% seawater at 10°C and 20°C. The ability to hyperosmotically regulate is present in the megalopa, and ontogenic changes occur in both ionic and osmotic regulation. First instar juvenile crabs, which are exposed to the greatest extremes of salinity and temperature in the field, are less able to osmoregulate than are the other three stages examined. Changes in Na⁺, Cl⁻, and K⁺ concentrations parallel total osmolality in all four stages. Hemolymph Mg⁺⁺ concentrations in megalopa and juveniles acclimated to 100% seawater are more than twice that of the concentration in the adult; after 8 h in 50% seawater, the megalopa and juvenile Mg⁺⁺ concentrations decrease to the level of the strongly regulated adult Mg⁺⁺ concentration. Ca⁺⁺ is strongly regulated by megalopas and adult crabs exposed to reduced salinity compared to the two juvenile stages. Diminished predation pressure and high food availability are proximate factors that may outweigh short-term osmoregulatory stress encountered on the tideflats during development of the juvenile crab.

Introduction

Estuarine invertebrates vary greatly in their abilities to deal with changes in ambient salinity. The effects of environmental salinity on the internal osmolality and specific ion regulation of adult estuarine crustaceans have

been investigated in numerous studies (for review, see Mantel and Farmer, 1983). Ontogeny of osmoregulation and ion regulation has been comprehensively studied in branchiopod crustaceans, especially the anostracan brine shrimp, *Artemia* (for review, see Conte, 1984). Comparable information about larval, post-larval, and juvenile decapod crustacean osmoregulation is relatively limited (Kalber, 1970; Foskett, 1977; Young, 1979; Read, 1984; Rabalais and Cameron, 1985; Charmantier *et al.*, 1988; Charmantier and Charmantier-Daures, 1991), and there are almost no data available regarding specific ion regulation during decapod crustacean development (Charmantier *et al.*, 1984a,b,c; Felder *et al.*, 1986).

The Dungeness crab, *Cancer magister*, inhabits the cold waters of the Pacific Northwest coast of North America and uses different portions of the estuarine and nearshore waters during its life cycle. Along the Oregon coast, embryos hatch from December through March (Reed, 1969; Lough, 1976). The newly hatched larvae go through five zoeal stages, all of which are planktonic in ocean waters, moving as far as 200 miles offshore. The transitional stage, an actively swimming planktonic megalopa, reenters the coastal and estuarine waters from mid April through early July (Lough, 1976). The megalopas then metamorphose into 1st instar juveniles that join the benthic community. Throughout the summer, juvenile crabs in the estuary are found in high numbers on the tideflats, while the adult crabs occur mainly in the deeper channels. Summer tidal changes in salinity and temperature, extending over a period of 6-8 h, are much greater on the tideflats than in the estuarine channels. Adults of *C. magister* do not migrate up into brackish waters for long periods as does *Callinectes sapidus*, the East and Gulf coast blue crab, but remain in the lower half of the bay, moving back and forth into nearshore waters.

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Previous studies on osmotic and ionic regulation in *Cancer magister* reported that adult crabs were weak hyperosmoregulators after 72–96 h exposure to dilute concentrations of seawater (Jones, 1941; Alspach, 1972; Engelhardt and Dehnel, 1973; Hunter and Rudy, 1975). These authors found that in reduced salinity, adult crabs were able to strongly hyporegulate magnesium and hyperregulate calcium. Hemolymph sodium, chloride, and potassium were hyperregulated but not as strongly as calcium. None of these studies examined the responses of larval, megalopa, or juvenile *C. magister* to changes in salinity.

In this paper, we ask how adults of *C. magister* respond osmotically to ecologically relevant short term tidal cycle changes in salinity. We also investigate whether the adult osmotic response pattern is present in the megalopa and juvenile stages or whether there is an ontogeny of osmotic regulatory abilities. Finally, we compare specific ion regulation during these life stages to see whether regulatory abilities for individual ions occur differentially during ontogeny.

Materials and Methods

Animals

Megalopas of *Cancer magister* (Dana) were collected with a dip net from the surface waters of Coos Bay, Oregon, from April through June of 1989. Because the megalopas molt within 48–72 h after collection, they were used in experiments within two days. In the laboratory, megalopas were kept in glass and wood aquaria (10 gallons) with running aerated seawater pumped on an incoming tide from near the mouth of Coos Bay. Salinity was 30–33‰, and water temperature was 10–12°C. Megalopas were not fed.

Juvenile crabs were raised from field-caught megalopas and were maintained in similar aquaria with running seawater and aeration. Adult males of *C. magister*, collected from the Coos Bay channel using crab pots, were kept in large holding tanks (260 gallons) with running seawater and aeration at the same temperature and salinity as megalopas and juveniles. Both juveniles and adults were fed 3–5 times a week on mussels, fish, and squid. Feeding was stopped 24 h prior to experiments to ensure a post-absorptive state in the crabs and to avoid their fouling the experimental chamber.

Protocol and sampling

Experiments were run on intermolt animals with juvenile intermolt stage based on time elapsed since the preceding molt. Thus, intermolt 1st instar juveniles were available in April–June, 5th instar juveniles in September–November, and adults in December–February.

Megalopas (approx. 3 mm carapace width), 1st instar juveniles (6–8 mm carapace width), 5th instar juveniles (25–33 mm carapace width), and adults (larger than 120 mm carapace width) were exposed to test conditions at varied temperatures and salinities for a period of 8 h. Hemolymph samples were taken immediately thereafter for osmotic and ionic analyses. Test conditions included 100% seawater (32‰, obtained on an incoming tide at the mouth of Coos Bay), 75% seawater, and 50% seawater (Coos Bay seawater diluted with glass distilled water) maintained at both 10°C and 20°C. Glass aquaria (one gallon) were used for the experimental chambers. About 250 megalopas or 1st instar juveniles and 2 or 3 5th instar juveniles were placed in each aquarium. Adults were kept one to an aquarium for the duration of the experiments.

Hemolymph was taken from the megalopas by puncturing the heart with a glass micro-capillary pipette. Juveniles and adults were bled by puncturing the arthrodistal membrane at the base of a walking leg; 1st instar juveniles were bled with micro-capillary pipettes, 5th instar juveniles and adults were bled with needle and syringe. Hemolymph obtained from all individuals in each experimental aquarium was pooled in order to collect a single sample of sufficient volume for both osmotic and ionic analyses. In figure legends 1–6, *n* refers to the number of separate pooled samples on which analyses were performed (megalopa, *n* = 1–3; 1st and 5th instar juveniles, *n* = 2–3; adult, *n* = 8). Seawater samples from each aquarium were collected. Samples were immediately frozen and stored at –73°C for subsequent osmotic and ionic analyses.

Osmotic and ionic analyses

Osmolality of seawater and hemolymph samples was measured using a Wescor 5500 vapor pressure osmometer. Chloride concentration was measured using a Buchler-Cotlove chloridometer. Magnesium concentration was measured colorimetrically after the method of Sky-Peck (1964). That is, samples were deproteinized with 5% trichloroacetic acid and reacted with thiazole yellow in the presence of excess base. The absorbance at 540 nm was measured with a Beckman DU-70 spectrophotometer. Sodium, calcium, and potassium ion activities were measured with a Radiometer Ion 83 ion meter in mV mode and the following electrodes: Radiometer G502 sodium Selectrode, Microelectrodes Inc. MI-420 sodium micro-electrode, Radiometer F2112 calcium Selectrode, and Orion 90-19 potassium electrode. The reference electrode in all cases was an Orion 90-02 double junction reference electrode with an NH₄Cl outer chamber filling solution and a AgCl saturated inner chamber filling solution. Samples were diluted 1:100 in the appropriate ionic strength adjustment solution. Prior to the analysis of samples, cal-

ibration for measurement of each ion species was done with salt solutions of known concentration spanning the expected range of values.

Data analysis

Results are expressed as mean \pm S.E. (n = number of observations). Three-way analysis of variance (ANOVA) was used to test for significance among treatments (developmental stage, salinity, and temperature). Subsequent multiple comparisons of means were performed using the Tukey-Kramer method. Statistical significance was accepted at $P < 0.05$.

Results

Estuarine salinity and temperature were measured in areas where the different developmental stages of *C. magister* were abundant in order to set limits for these parameters in laboratory studies. The tidelflat environment of juveniles ranges from 10°C at high tide to 25°C when the tide has receded and tidelflats are exposed during early- to mid-morning low tides in summer. At the same time, salinity drops from 32 to 16‰ as the freshwater lens on the surface passes down the flats. In the channels where adults are found, summer water temperature (10–15°C) and salinity (32–20‰) are much more stable. Winter water temperature is consistently low (10–12°C). Winter range of salinity (32–16‰) at depth in the estuary varies as widely as salinity on the summer tidelflats owing to the increased fresh water input from rain.

Osmoregulation

After 8-h exposure to 100% seawater, the megalopa, 1st instar juvenile, 5th instar juvenile, and adult are isosmotic with the ambient seawater (Fig. 1). In 75% seawater, the hemolymph osmolalities of all four stages are significantly lower than in 100% seawater, yet they are all hyperosmotic relative to 75% seawater. In 50% seawater, the hemolymph osmolalities of all four stages are significantly lower than in 75% seawater, and all are significantly hyperosmotic compared with 50% seawater. The 1st juvenile is least able to maintain hemolymph osmolality in dilute seawater compared to the other stages examined. The crabs are less able to osmoregulate in warmer water. Hemolymph osmolalities of the adult and 5th instar juvenile are significantly lower at 20°C than at 10°C in both 75% and 50% seawater; megalopa hemolymph osmolality is also lower at 20°C than at 10°C in 50% seawater.

Ionic regulation

The hemolymph chloride concentration in all four stages in 100% seawater is hypoionic compared with ambient seawater (Fig. 2). In 75% seawater the adult becomes

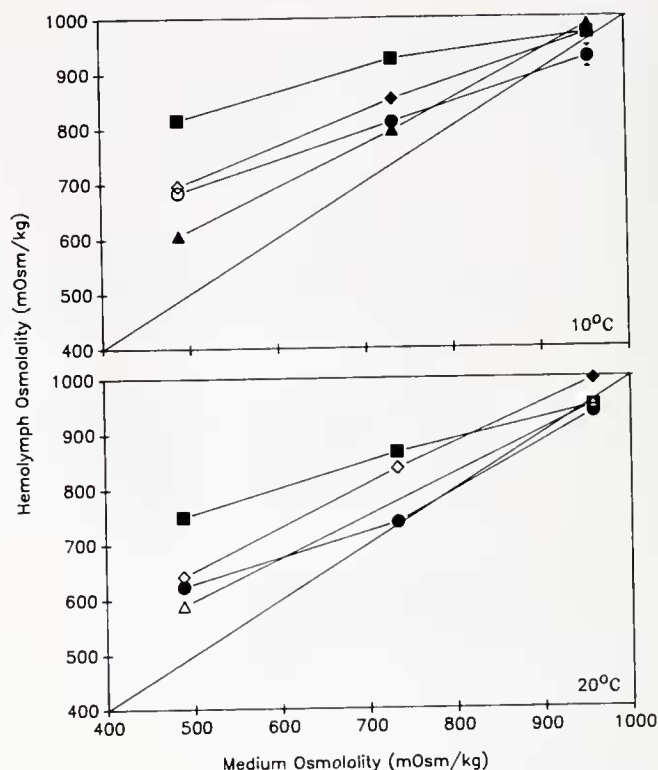


Figure 1. Hemolymph osmolality of *Cancer magister* as a function of medium osmolality for \diamond , megalopa ($n = 1-3$); \blacktriangle , 1st instar juvenile ($n = 2-3$); \bullet , 5th instar juvenile ($n = 2-3$); \blacksquare , adult ($n = 8$). Solid symbols, $n > 2$, standard error bars drawn; open symbols, $n \leq 2$, mean.

nearly isoionic compared with the seawater and has significantly lower hemolymph chloride concentration at 20°C than at 10°C. In 50% seawater the adult hemolymph chloride concentration is hyperionic and is lower at 20°C than at 10°C. The hemolymph chloride concentrations of the megalopa and of the 5th instar juvenile are also temperature sensitive in 75% seawater. In 50% seawater the megalopa and 1st instar juvenile hemolymph chloride concentrations are the same as the ambient seawater chloride, while that of the 5th instar juvenile is significantly higher than that of the megalopa and 1st instar juvenile and lower than that of the adult.

Hemolymph sodium ion activity (Fig. 3) in all four stages shows essentially the same pattern as hemolymph chloride. In 100% seawater all four stages are hypoionic with respect to ambient seawater sodium. In 75% seawater the megalopa, 1st instar juvenile, and 5th instar juvenile hemolymph sodium ion activities are significantly less than in 100% seawater, while the adult hemolymph sodium ion activity is not significantly changed. In 50% seawater the megalopa and 1st instar juvenile hemolymphs are isoionic to ambient sodium, whereas the 5th instar juvenile hemolymph sodium is intermediate between the younger stages and the adult. There is no significant effect

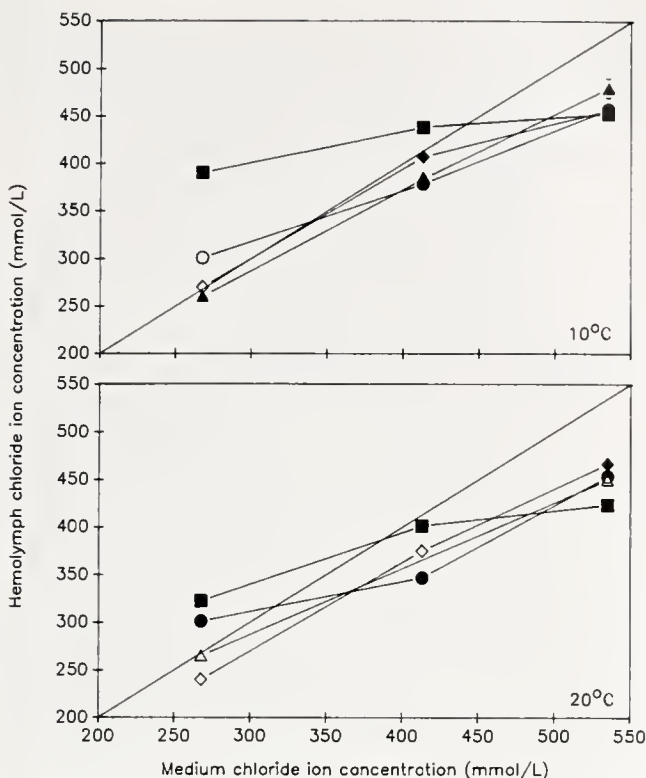


Figure 2. Hemolymph chloride ion concentration of *Cancer magister* as a function of medium chloride ion concentration for \blacklozenge , megalopa ($n = 1-3$); \blacktriangle , 1st instar juvenile ($n = 2-3$); \bullet , 5th instar juvenile ($n = 2-3$); \blacksquare , adult ($n = 8$). Solid symbols, $n > 2$, standard error bars drawn; open symbols, $n \leq 2$, mean.

of temperature on the hemolymph sodium ion activity in any of the stages.

There is no significant effect of temperature on hemolymph potassium ion activity (Fig. 4). The megalopa shows no significant change in hemolymph potassium ion activity. The adult, 1st instar juvenile, and 5th instar juvenile hemolymph potassium ion activities, however, are significantly less in 75% and 50% seawater than in 100% seawater.

In contrast to the concentrations of chloride, sodium, and potassium, that of magnesium is strongly hyporegulated in adult hemolymph in all salinity treatments (Fig. 5). In 100% seawater the megalopa, 1st instar juvenile, and 5th instar juvenile hemolymph magnesium concentrations are significantly higher than the adult. As salinity decreases, magnesium concentration in these three stages also decreases until, in 50% seawater, there is no difference in the hemolymph magnesium concentration among all four stages. The only stage in which magnesium regulation shows a significant temperature sensitivity is the 5th instar juvenile in 100% seawater.

In 100% seawater the hemolymph calcium ion activities in all four stages are not significantly different from the

ambient seawater calcium ion activity (Fig. 6). The hemolymph calcium ion activities of the megalopa and adult do not change significantly with salinity. The 1st instar juvenile and 5th instar juvenile, however, have significantly lower hemolymph calcium ion activities in 75% and 50% seawater than in 100% seawater. Overall there is no significant effect of temperature on hemolymph calcium ion activity.

Discussion

Different developmental stages in the life cycle of *Cancer magister* have distinctly different patterns of hemolymph osmotic and ionic regulation when exposed to reduced salinity. The values for hemolymph osmolality and ionic concentrations in the present study were obtained after an 8-h exposure time, the duration of a tidal cycle, which is physiologically and ecologically relevant for these crabs. Furthermore, the general trends for osmotic and ionic regulation reported in the long-term 72–96 h equilibrium exposures (Jones, 1941; Alspach, 1972; Engelhardt and Dehnel, 1973; Hunter and Rudy, 1975) are apparent after the 8-h exposure time used here. We find, just as the

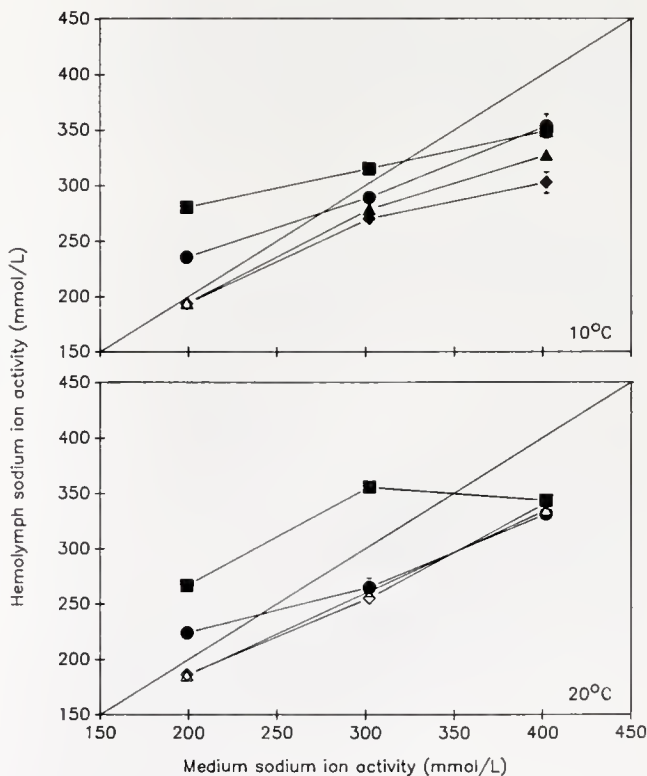


Figure 3. Hemolymph sodium ion activity of *Cancer magister* as a function of medium sodium ion activity for \blacklozenge , megalopa ($n = 1-3$); \blacktriangle , 1st instar juvenile ($n = 2-3$); \bullet , 5th instar juvenile ($n = 2-3$); \blacksquare , adult, ($n = 8$). Solid symbols, $n > 2$, standard error bars drawn; open symbols, $n \leq 2$, mean.

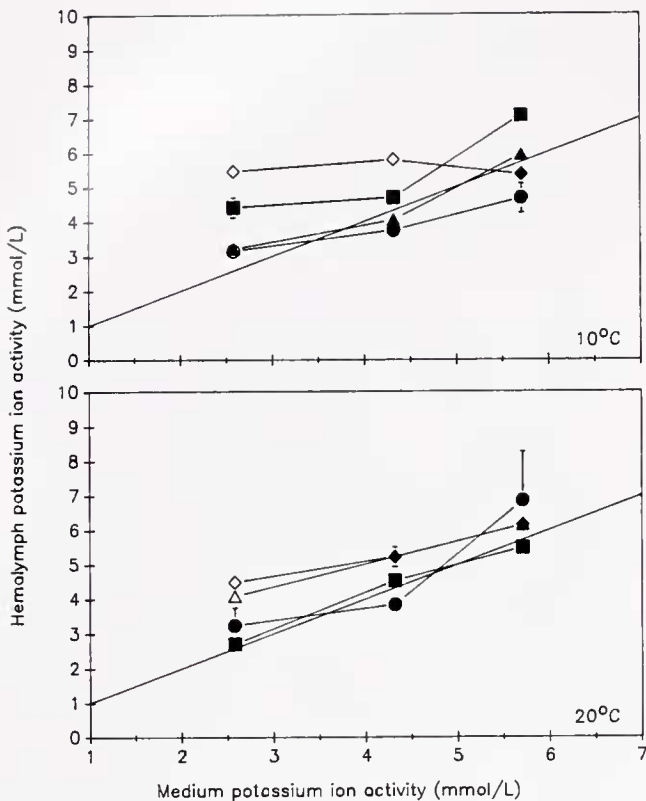


Figure 4. Hemolymph potassium ion activity of *Cancer magister* as a function of medium potassium ion activity for \diamond , megalopa (n = 1–3); \blacktriangle , 1st instar juvenile (n = 2–3); \bullet , 5th instar juvenile (n = 2–3); \blacksquare , adult (n = 8). Solid symbols, n > 2, standard error bars drawn; open symbols, n ≤ 2, mean.

earlier equilibrium studies reported, that the hemolymph of adult *C. magister* is weakly hyperosmoregulated in water less concentrated than normal ocean seawater; chloride, sodium, and potassium are somewhat hyperregulated in reduced salinity, magnesium is very strongly hyporegulated and calcium is strongly hyperregulated. Compared with adults of four other species within the genus *Cancer* for which data are available (see Charmantier and Charmantier-Daures, 1991, for review), *C. magister* adults are the strongest osmoregulators. For example, *Cancer antennarius* has a hemolymph osmolality only 15 mOsm/kg above ambient seawater osmolality in approximately 53% seawater at 15–20°C (Jones, 1941) compared with *C. magister* hemolymph osmolality of 250 mOsm/kg above ambient seawater in 50% seawater at 20°C.

Ontogeny of osmoregulation

Studies on the larvae, post-larvae, and juveniles of a number of decapod crustacean species indicate that most larvae and post-larvae can maintain hemolymph osmolality above that of ambient seawater, either by hyperos-

moconforming or by weakly hyperosmoregulating (see Charmantier *et al.*, 1988). In such cases, metamorphosis often marks a profound change in osmoregulation from larval to adult patterns. Many decapods that are hyperosmoconforming or weakly hyperosmoregulating over a wide range of salinities in the premetamorphic stages undergo a change to become either (a) strongly hyperosmoregulating in low salinity and osmoconforming in high salinity in the adult, as in *Clibanarius vittatus*, *Homarus gammarus*, *Homarus americanus*, and *Cancer irroratus* (Young, 1979; Thuet *et al.*, 1988; Charmantier *et al.*, 1988; Charmantier and Charmantier-Daures, 1991) or (b) hyper-hypoosmoregulating in the adult, as in *Sesarma reticulatum*, *Uca subcylindricum*, and *Penaeus japonicus* (Foskett, 1977; Rabalais and Cameron, 1985; Charmantier *et al.*, 1988). Less frequently described are species in which the larvae have a greater osmoregulatory ability than the adult, *i.e.*, *Hepatus epheliticus* and *Libinia emarginata*, but a switch still occurs around the time of metamorphosis (Kalber, 1970). *Macrobrachium petersi* is a case in which both larvae and adults are strong osmoregulators, but the different stages vary in their capacity to hypo or hyperosmoregulate (Read, 1984). To date the

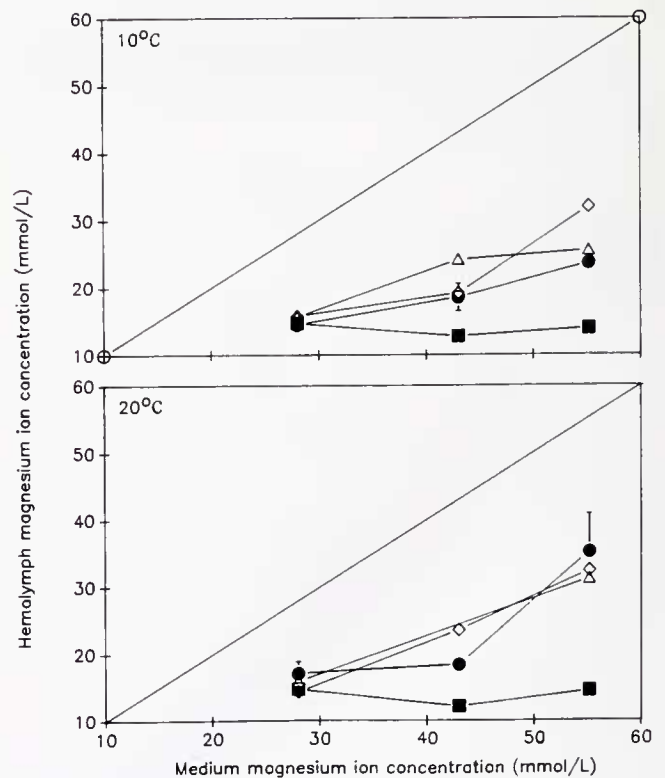


Figure 5. Hemolymph magnesium ion concentration of *Cancer magister* as a function of medium magnesium ion concentration for \diamond , megalopa (n = 2); \blacktriangle , 1st instar juvenile (n = 2); \bullet , 5th instar juvenile (n = 2–3); \blacksquare , adult (n = 8). Solid symbols, n > 2, standard error bars drawn; open symbols, n ≤ 2, mean.

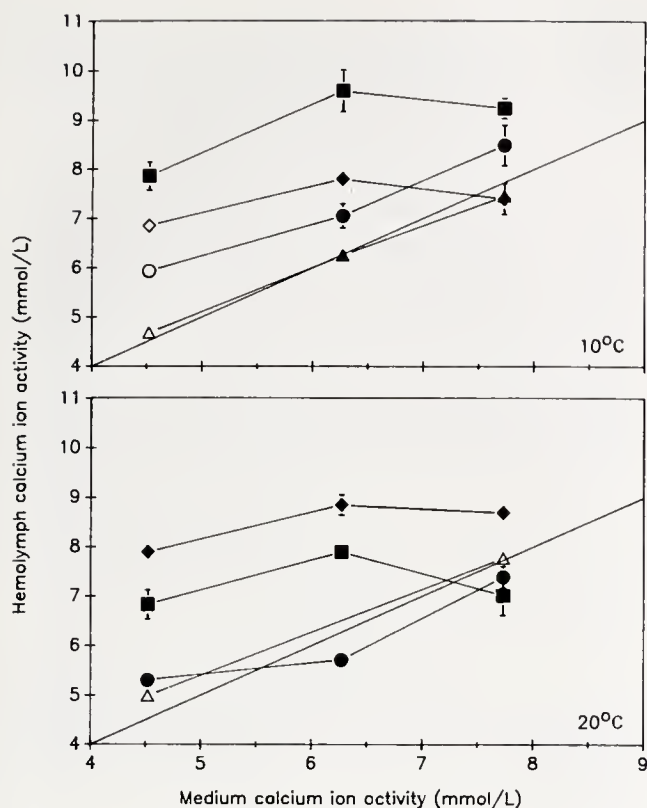


Figure 6. Hemolymph calcium ion activity of *Cancer magister* as a function of medium calcium ion activity for \blacklozenge , megalopa ($n = 1-3$); \blacktriangle , 1st instar juvenile ($n = 2-3$); \bullet , 5th instar juvenile ($n = 2-3$); \blacksquare , adult ($n = 8$). Solid symbols, $n > 2$, standard error bars drawn; open symbols, $n \leq 2$, mean.

only decapod species whose osmoregulatory pattern does not change during development is *Callinassa jamaicensis* var. *louisianensis*. Its larvae remain in the hypersaline burrow habitat of the adult, and all stages show limited hyperosmoregulation in dilute media, although the adult can hyperregulate a bit more strongly than the zoeae (Felder, 1978; Felder *et al.*, 1986).

In the present study, there is a marked change in osmoregulation at metamorphosis from megalopa to 1st instar juvenile in *C. magister*. Interestingly, the juvenile *C. magister* is less able to regulate over the short 8-h exposure than is the megalopa (Fig. 1). As development proceeds, however, osmoregulatory ability becomes more like that of the adult. A correlation between ontogeny of osmoregulation and changes in habitat salinity has been observed for several species (for review, see Charmantier *et al.*, 1988). This correlation does not hold as an explanation for the observed patterns of osmoregulation in the different stages of *C. magister*. The 1st instar juvenile, the stage least able to osmoregulate, is found in high numbers on the mudflats where it encounters extremes of low salinity and high temperature. Other factors, such as the short

duration of exposure to these extremes and behavioral responses, must play a role in environmental distribution. Both juvenile and adult *C. magister* become inactive in low salinities. This behavior may enable the juveniles to endure the low salinity portion of the tide cycle on the mudflat without great expenditure of energy while experiencing protection from heavy predation by adult crabs and fish concentrated during low tide in the deeper channels. As the tide rises, juveniles on the mudflats are able to immediately resume active foraging; proximity to high food availability may offset the short term osmoregulatory stresses experienced during low tide.

Several factors may be responsible for the diminished ability of the 1st instar juvenile to osmoregulate. Although megalopas, 1st, and 5th instar juveniles all have gills that function in ion transport based on silver staining (Brown, unpub. obs.), there are no data available on the ratio of gill surface area to total body volume in these stages. The carapace of the 1st instar juvenile is about twice as wide as in the megalopa, and the juvenile weighs twice as much; if the gill surface area has not increased proportionately, this might explain the diminished osmoregulatory capacity of the juveniles. Also important are the efficiency of salt transport at the gill and the amount of area on the gill associated with that salt transport (see Conte, 1984). Felder *et al.* (1986) have shown differences in Na^+/K^+ ATPase activity in the different pre-hatch stages of *Callinassa jamaicensis* var. *louisianensis* and have demonstrated the presence of salt transport type tissue on the brancho-stegites of the zoeae. *Homarus gammarus* post-larvae, which have greater osmoregulatory capacities compared with larval stages, show a marked increase in gill Na^+/K^+ ATPase and carbonic anhydrase activities (Thuett *et al.*, 1988). It is possible that the juvenile stages of *C. magister* initially have lower Na^+/K^+ ATPase activity levels or a different relative proportion of salt transporting tissue than the megalopas.

Ontogeny of ion regulation

This is the first report of an ontogenic change in specific ion regulation in brachyuran crabs. The data show that specific ions are regulated differently by megalopa, juvenile, and adult *C. magister*. At metamorphosis from megalopa to 1st instar juvenile, there are shifts in specific ion regulatory patterns that do not parallel changes in hemolymph osmolality. Ion regulation in 5th instar juvenile is more like that in adult than that in 1st instar juvenile; the fifth instar juvenile is beginning to show the adult pattern of ion regulation but does not regulate to the same extent as the adult.

Hemolymph levels of sodium, chloride, and potassium parallel stage-specific changes in hemolymph osmolality as salinity decreases. Sodium and chloride are the two

main inorganic ion constituents in the hemolymph and appear to be the major components in osmotic regulation in the different developmental stages.

Two aspects of developmental changes in specific ion regulation are particularly striking. First, hemolymph magnesium concentrations in megalopas and in 1st and 5th instar juveniles in 100% seawater are twice as high as in the adult, when none of the other ions show any differences between stages in 100% seawater. Second, calcium is strongly hyperregulated in megalopa and adult hemolymph, as salinity decreases, compared to the two juvenile stages studied.

Adults of all species of crustaceans that have been examined maintain hemolymph magnesium well below the magnesium concentration of the ambient water, except when they are in extremely dilute water. Engelhardt and Dehnel (1973) stated that "hyporegulation of magnesium is the most universal feature of ionic regulation in crustacean blood." Adult *C. magister* excrete magnesium in urine formed in the antennal gland; the urine to hemolymph ratio of magnesium is nearly 4:1 in 100% seawater (Hunter and Rudy, 1975; Holliday, 1980). In the early stages of crustaceans, the antennal gland may not be fully developed and functional (Waite, 1899; Conte, 1984), and this may account for the high hemolymph magnesium in megalopa and juvenile crabs. Low hemolymph magnesium levels have often been associated with high levels of activity or a greater extent of terrestriality in crustaceans. According to Robertson (1960), decapod species with hemolymph magnesium concentrations less than 50% that of seawater are more active than those with higher hemolymph magnesium concentrations. In fact, high magnesium concentrations are often used to anaesthetize marine invertebrates. The totally aquatic *C. magister* megalopa is an extremely active animal, however, capable of swimming very rapidly for extended periods. The 1st instar juvenile, which like the megalopa has more than twice the adult's hemolymph magnesium, is also considerably more active than the adult. Gross (1964) discusses magnesium regulation at length in relation to the extent of terrestriality of various crab species. Mantel and Farmer (1983) note that grapsids and other species of semi-terrestrial and terrestrial decapods all have low hemolymph magnesium concentrations. Because all stages in the life cycle of *C. magister* are aquatic, the change from higher to lower magnesium concentration we observe during development is not related to changes in extent of terrestriality. In summary, the high magnesium in the hemolymph of the megalopa and juvenile and the developmental changes in magnesium levels are consistent with neither of these hypotheses, activity level or terrestriality.

Calcium regulation in *C. magister* adults has been previously reported (Alspach, 1972; Engelhardt and Dehnel, 1973; Hunter and Rudy, 1975). The strong regulation of

calcium by the megalopa and weak regulation by the juveniles is noteworthy. The low levels of calcium in the hyposaline-exposed 1st and 5th instar juveniles may reflect their overall decreased ability to regulate ions.

In summary, tidal cycle changes in salinity and temperature have a strong effect on hemolymph osmolality and ionic concentration in megalopas, juveniles, and adults of *C. magister*. There are ontogenic changes in both ionic and osmotic regulation in *C. magister*. Calcium and magnesium regulation change markedly during development from megalopa to adult crab. Both of these ions have strong effects on the oxygen affinity and the cooperativity of hemocyanin from a variety of crustacean species (Larimer and Riggs, 1964; Miller and Van Holde, 1974; Truchot, 1975). The changes in hemolymph calcium and magnesium levels during the development of *C. magister* may be involved in modulating the oxygen-binding properties of the hemocyanin. This hypothesis is currently under investigation.

Acknowledgments

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